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Allelopathic Effect of Macroalga *Gracilaria Tenuistipitata* (Rhodophyta) on the Photosynthetic Apparatus of Red-tide Causing Microalga *Prorocentrum micans*

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Abstract

Under controlled indoor conditions, the red-tide causing microalga, *Prorocentrum micans* was co-cultured with varied grams of sheared macroalga *Gracilaria tenuistipitata* (Rhodophyta) in the coexistance culture system for three days, to characterize the mitigant roles by the seaweed in photosynthesis of the microalga. The polyphasic rising phase of fluorescence transients displayed by the oxygenic photosynthetic organisms at the initial illumination stage are named as OJIP. In this study, the transient flourescence curves of chlorophyll *a* with relevant parameters in *P. micans* were tracked precisely and clarified based on tests of JIP parameters to analyze photosystem II activity. The photosynthetic mitigant roles on *P. micans* by dried *G. tenuistipitata* on were obvirously gram- and time-relevant. Chl *a* fluorescence specific parameters followed the same patterns as the O-J-I-P profiles. Based on the transient flourescence curves with their relevant parameters, the main inhibitory sites by *G. tenuistipitata* on the photosynthetic appartus of *P. micans* might be listed as the decline in active numbers of photosystem reactive centers, coupled with blockng-up of the electron-transporting chain. From results of present study, it is clear that dried segments of *G. tenuistipitata* thalli have effectively inhibitory effect on photosynthesis of *P. micans*, indicating the potential algicide sources of the dried *G. tenuistipitata* for use against *P. micans* causing red tide.

Keywords: Hamful algal blooms (HABs); Gracilaria tenuistipitata; Prorocentrum micans; Allelopathy; JIP-test

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1. Introduction

Urbanization, aquaculture and anthropogenic discharge have resulted in nutrient enrichment (namely, eutrophication) in many estuarine and coastal waters, especially in the developed area (Anderson et al., 2008). Eutrophication and consequent explosive growth of microalgae whether in toxic or nontoxic forms, which is named as red-tide or harmful algal blooms (HABs), has been considered to be the key ecological issues in coastal waters during the most recent two to three decades (Heisler et al., 2008; Lewitus et al., 2012).

Prorocentrum micans, capable of foming blooms, is a HABs species, and can cause shellfish morality (Wang et al., 1998). In order to control and/or mitigate HABs, different physical approaches, such as light-shading and solar ultraviolet radiation (Sugawara et al., 2003; Chen et al., 2009), and chemical ways (Sun and Choi, 2004; Lee et al., 2008) have been developed. However, due to the ecological and cost account, the large-scale applications of the above mentioned ways are confined (Wang et al., 2009). Biological controls using macroalgae, examples of *Ulva pertusa* and *Gracilaria* are found able to weaken HABs effectively, and also are indigenous to the marine environment, with other merits of easy collecion, low cost and environmental benignancy (Jin and Dong, 2003; Tang and Golber, 2011; Wang et al., 2012). *G. tenuistipitata*, distribued widely in brackish areas, is a useful macroalga for agar, biological active species and fodder additive for aquaculture (Xu and Gao, 2008; Zheng and Gao, 2009; Qi et al., 2010; Yeh et al., 2012). It's proved that the genus of *Gracilaria* and other macroalgae have an obvious weaken effect on the proliferation of some HABs, whether as water-soluble extract, culture filtrate, dry powder or fresh thalli (Wang et al., 2007; Nan et al., 2008; Oh et al., 2010). By changing the dominant species and reducing phytoplankton abundance, macroalgae can mitigate the detrimental effects of HABs, selectively recompose the community of phytoplankton (Zhou et al.,2006; Ye et al., 2012). Compared with the direct use of fresh macroalgal thalli to control and/or mitigate the HABs, the use of macroalgal extract or dried powder is more predominant, as their concentrations are easily controlled in application, and thus much safer. However, little is known about the profiles of photosynthetic inhibition against HABs by the dried seaweed.

In this study, we use coexistence culture systems to elucidate the photosynthetic inhibition patterns on *P. micans* by dried *G. tenuistipitata*. Characterizing these patterns can explain the mechanisms of using dried *G. tenuistipitata* as as the potential algicide sources against *P. micans* causing red tide.

2. Materials and methods

2.1. Collected site and pre-culture of the macroalga

In September 2012, *G. tenuistipitata* was collected from the Hengqin Island, Zhuhai, Guangdong, China. The macroalga were flushed instantly with filtered seawater to clear unwanted materials from the thalli surface, and then pre-cultured for acclimatization about a week. The pre-culture conditon is set as below: irradiance of about 40 μmol photons m^{-2} s^{-1}, temperature of 20 ± 0.1°C, and daily rhythmicity of 12 h.

2.2. Culture of G. tenuistipitata after pre-culture

The macroalga was kept growing in the sea-water with 7 μmol L^{-1} NaH_2PO_4 and 100 μmol L^{-1} $NaNO_3$ as enrichment indoor for a week, before the co-culture experiment was made with microalga. The culutred conditon is set as following: irradiance of about 70 μmol photons m^{-2} s^{-1}, temperature of 20 ± 0.1°C, and nightly rhythmicity of 12 h.

2.3. Culture of P. micans

P. micans was inoculated in f/2 medium with modification (Guillard, 1975) under same condition as *G. tenuistipitata*. The salinity of the final medium was 30‰, with pH being 8.0. The flask with *P. micans* was shaken handly two times perday. The microalga was kept growing to the exponential stage for use in the co-culture experiments. In 500 ml Erlenmeyer flasks, certain volume of *P. micans* was inoculated into 350 ml sea-water with f/2 medium enrichment. Initial incoulalted concentration of microalga was 1 x 10^4 per millilitre.

2.4. Co- cultrure experiments with P. micans and dried G. tenuistipitatao

To preapre the dried thalli of *G. tenuistipitata*, the fresh macroalga was firstly removed the salt by rinsing with filtered seawater, and then make it dry with air. The dry sample was fragmented by shearing, the final biomass was set as 0, 1.0, 2.0 and 3.0 g dry weitht per litre. Different biomass of sheared *G. tenuistipitata* were co-cultured with *P. micans* in flasks. were labelled The control was monocultures with only microalga of *P. micans*. Co-culture experiments proceeded for 3 days with three replicates.

2.5. Determination of Chlorophyll Fluorescence and JIP-test

During the co-culture experiment, the fluorescence transients state of Chl *a* in *P. micans* were tracked by an analyser for plant efficiency (Hansatech Instruments, England). Before measuring, all samples should be dark -adapted for 15 min in a black bag. The polyphasic rising phase of fluorescence transients, which are displayed by the oxygenic photosynthetic organisms at the initial illumination, are named as OJIP (Strasser et al., 1995; Appenroth et al., 2001). The detailed information about determination of Chlorophyll fluorescence and JIP-test can refer to the paper by Ye et al. (2012). In this study, OJIP cures of *P. micans* and three relevant parameters, RC/Cso (at t=0, the active number in photosystem reactive center per excited cross-section), F_v/F_m (the PSII maximum photochemical efficiency) and ETo/Cso (at t=0, electron transporting flux per excited cross-section) are used to appraise the controlling effect of dried macroalga *G. tenuistipitata* on the photosynthetic apparus of microalga *P. micans*.

2.6. Statistical analysis of Data

Data statistics were performed by one-way ANOVA in SPSS 13.0, with $P< 0.05$ being significant.

3. Results

The dried biomass of *G. tenuistipitata* had a strongly stimulative or algicidal effects on *P. micans*, depending on the biomass of dried macroalga (Fig. 1).

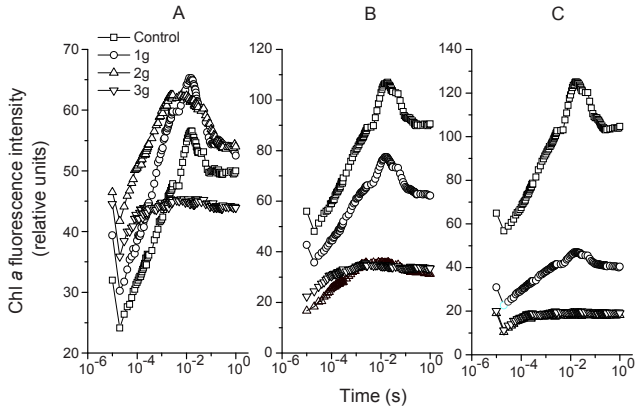

Fig. 1. The OJIP profiles of *P. micans* co-existed with scaled grams of dried *G. tenuistipitata* during three days experiment (A, first day; B, sencond day; C, third day).

On the first day, compared with control Chl *a* fluorescence intensity of *P. micans* was first significantly simiulated by 1g and 2g dried *G. tenuistipitata* ($P<0.01$), and then heavily restrained by 3g dried macroalga ($P<0.01$), inhibiton of which was also concentration-relevant in latst two days (Fig. 1).

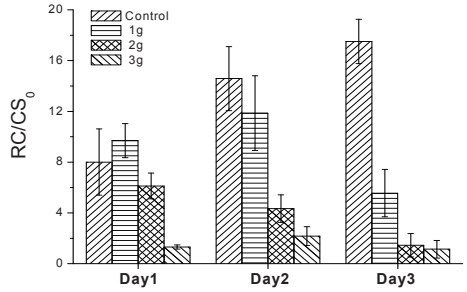

Fig. 2. RC/CSo of *P. micans* co-existed with scaled grams of dried *G. tenuistipitata* duing experiment.

The higher grams of dried *G. tenuistipitata* heavily lowered the RC/CSo of *P. micans* both on day1, day2 and day3, and the mitigant effect was gram relevant (Fig. 2).

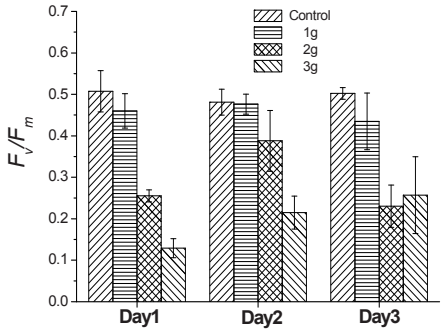

Fig. 3. The PSII maximum photochemical efficiency of *P. micans* co-cultured with scaled grams of dried *G. tenuistipitata* duing

experiment.

The same clear concentration-depenent stimulative or inhibitory pattens by dried *G. tenuistipitata* on *P. micans* were followed by F_v/F_m and ETo/CSo during the experiment (Fig.3, Fig.4).

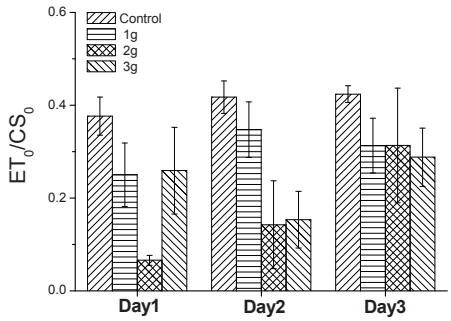

Fig. 4. ETo /CSo of *P. micans* co-cultured scaled grams of dried *G. tenuistipitata* duing experiment.

4. Discussion

Our experiment proved that dried macroalga *G. Tenuistipitata* had effectively inhibitory effect on the photosynthesis of microalga *P. micans*. Tand and Golber (2011) also found that the dried *U. lactuca* had advantage over its equivalent fresh thalli in mitigating the growth of HABs equally or more potentially. The active allelopathic effects could attribute to organosulfur compounds or poly-PUFA (Alamsjah et al., 2008). By making experiment on microalga, Wium-Andersen et al. (1982) proved that some other iosolated and identified chemicals from *Characean* species, such as dithiolane and trithiane compounds have allelopathic effects on microalgae. Different extracts of macroalgae have more intensive potency in inhibiting growth of microalgae compared with fresh macroalgal samples, which indicates that allelochemicals are congregated more in cell than being released into the water. From the macroalga *G. lemaneiformis*, the same genus of *G. tenuistipitata*, some metabolins, such as linoleic acid, palmitic acid and 6E-octadien-3-one were isolated by Lu et al. (2011), and proved to have obviously allelopathic role in the proliferation of HABs species of *Skeletonema costatum*. Futhermore, in our study, we sheared the dried *G. tenuistipitata* thalli by scissors, which may result in more allelochemicals being released to the environment (Reigosa et al., 1999).

In our study, the O-J-I-P curve of *P. micans* was firstly simiulated by lower grams of dried *G. tenuistipitata*, and then inhibited by higher grams of dried macroalga. A clear concentration-dependent stimulative or inhibitory profile was characterized between the gram of *G. tenuistipitata* and its relevant effect on *P. micans*. This result was consistent with other studies which indicated that certain allelopathic agents had positive or negative effects on targets based on corresponding lower or higher concentrations (Van Aller et al., 1998). According to the characterization of Chl *a* transient fluorescence curves and their relevant parameters (RC/CS_0, F_v/F_m and ETo /Cso), the electron transport and oxygen-evolving complex of *P. micans* were destroyed and/or blocked by the higher grams of dried *G. tenuistipitata*, which finally resulted in the decrease of photosynthesis in *P. micans*.

Our resluts were consistent with the results achieved by Zhu et al. (2010), who made experiment with macrophyte *Myriophyllum spicatum*, and found that the allelochemicals such as polyphenols, pyrogallic acid and gallic acid isolated from submerged organism were key agents in inhibiting the photosyntheitc activity of *Microcystis aeruginosa*r. Based on the proof from oxygen evolution complex to the photooxidized

chlorophyll, and proof from the oxygen evolution complex to PQ, the inhibition sites by *M. spicatum* lie in PSII and the whole chain of *M. aeruginosa*r (Zhu et al. 2010). In our results by anaylzing the chlorophyll fluorescence kinetics and their relevant parameters, the prime photosynthetic suppressive locus of *G. tenuistipitata* on *P. micans* might be due to a declined active reaction centers numbers and electron transport chain blocking in photosystem of *P. micans*.

5. Conclusion

The present study demonstrated that the dried *G. tenuistipitata* imposed an obviously mitigant role in the photosynthesis of *P. micans*, and might be a potential algicide sources against *P. micans* causing red-tide.

Acknowledgements

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